Technical Data Sheet

# Purified Mouse Anti-eNOS (pT495)

## Product Information

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|---|---|
| **Material Number:** | **612706** |
| **Size:** | 50 µg |
| **Concentration:** | 250 µg/ml |
| **Clone:** | 31/eNOS(pT495) |
| **Immunogen:** | Human Phosphorylated eNOS Peptide |
| **Isotype:** | Mouse IgG1 |
| **Reactivity:** | QC Testing: Human<br>Tested in Development: Mouse, Rat, Dog |
| **Target MW:** | 140 kDa |
| **Storage Buffer:** | Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide. |

## Description

Nitric oxide synthase (NOS), a cell-type specific enzyme, catalyzes the synthesis of nitric oxide (NO). NO is a short-lived radical that transmits signals involved in vasorelaxation, neurotransmission, and cytotoxicity. In neurons and endothelial cells, constitutive NOS (cNOS) is activated by agonists that increase intracellular Ca2+ levels and enhance calmodulin binding. Neuronal NOS (nNOS) and endothelial NOS (eNOS) have recognition sites for NADPH, FAD, FMN, and calmodulin. eNOS has a unique N-myristylation consensus sequence that may explain its membrane localization. Various protein kinases have been implicated in regulation of eNOS activity, including AMPK, PKA, PKB/Akt, PKC, and CaM Kinase II. During VEGF stimulation, eNOS is transiently phosphorylated at Ser-1177 by PKB/akt and dephosphorylated at Thr-495. At later time points, VEGF stimulation leads to an increase in Thr-495 phosphorylation mediated by PKC and a decrease in Ser-1177 phosphorylation. In addition, Ser-495, Ser-633, and Ser-1177 are phosphorylated by PKA and PKG in vitro. Thus, eNOS activity may be regulated through complex phosphorylation events mediated by multiple kinases at various phosphorylation sites.

## Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at -20°C.

## Application Notes

**Application**

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| Western blot | Routinely Tested |

**Recommended Assay Procedure:**

***Western blot:*** Please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

***Western blot analysis of eNOS (pT495).*** *A human endothelial cell lysate (lane 1), recombinant eNOS protein before in vitro phosphorylation of Thr-495 (lane 2), and recombinant eNOS protein after in vitro phosphorylation of Thr-495 with PKA kinase (lane 3) was probed with a 1:1000 dilution of the mouse anti-eNOS (pT495) antibody. eNOS (pT495) may be observed to be migrating at ~ 140 kDa.*

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## Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|---|---|---|---|
| 611450 | Human Endothelial Cell Lysate | 500 µg | (none) |
| 554002 | HRP Goat Anti-Mouse Ig | 1.0 ml | (none) |

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

## References

Michell BJ, Chen Zp, Tiganis T, et al. Coordinated control of endothelial nitric-oxide synthase phosphorylation by protein kinase C and the cAMP-dependent protein kinase. *J Biol Chem.* 2001; 276(21):17625-17628.(Biology)